

During a survey of parasitic helminths of wild vertebrates from Tres Palos Lagoon, in Guerrero, Mexico, we found *Gnathostoma* sp. advanced third-stage larvae (AdvL<sub>3</sub>) in the skeletal muscle of several fish species. Fish were caught from March to August 1999 in Tres Palos Lagoon (16° 41' to 16° 50'N and 99° 37' to 99° 47'W), Acapulco Municipality, 25 km south of Acapulco Bay (5). Fish muscle was ground individually, compressed between glass plates, and examined with a magnifying glass and a lamp. The infection was characterized as by Margolis et al. (6).

Of nine fish species examined, five were positive for *Gnathostoma* AdvL<sub>3</sub>: Eleotridae: *Dormitator latifrons* ("popoyote," n = 83), *Gobiomorus maculatus* ("guavina," n = 66), *Eleotris pictus* ("alahuate," n = 22); Cichlidae: *Cichlasoma trimaculatum* ("charra," n = 62), and Ariidae: *Cathorops caerulescens* ("cuatete," n = 62). The highest prevalence and mean abundance values (number of larvae per fish) were found in *E. pictus* (31.81%, 0.82 ± 1.99); in the other host species values were ≤7.22 and 0.072 ± 0.26, respectively. *E. pictus* mean abundance values differed significantly from those of the other host species (nonparametric Kruskal-Wallis test, H = 27.125, 4 g.l., n = 337, p < 0.0).

The intermediate host transmitting the infection to humans in Mexico had previously been identified only in the Rio Papaloapan Basin, in Veracruz and Oaxaca (7,8). The presence of *Gnathostoma* AdvL<sub>3</sub> in the muscle of fish species frequently eaten by humans in Acapulco suggests that these fish may have been the main source of infection in the 98 recorded cases of gnathostomosis (3,4). The popularity of "ceviche" (raw fish marinated in lime juice), prepared with the most commonly caught fish (including the three species of eleotrids studied), strongly supports this possibility. The identification of the source of human infection allows local health authorities to implement public information campaigns about the risk of eating raw or undercooked fish (in the form of sushi or ceviche) in this region. After this initial step in the study of this parasitic disease, the worm species must be accurately identified. In addition, understanding the parasite's life cycle is important for control of a parasitic disease.

## Acknowledgments

We thank F. Bertoni, E. Martínez, C. Castilla, A. Monet, E. Cabrera, and B. Mendoza for their help in field collections.

This study was supported by project PAPIIT-UNAM IN219198 to VL-R.

**Virginia León-Règagnon, Luis García-Prieto,  
David Osorio-Sarabia, and  
Agustín Jiménez-Ruiz**

Universidad Nacional Autónoma de México,  
México D.F., México

## References

1. Peláez D, Pérez-Reyes R. Gnathostomiasis humana en América. *Rev Latinoam Microbiol* 1970;12:83-91.
2. Lamothe AR, Osorio SD. Estado actual de la gnathostomiasis en México. *Anales del Instituto de Biología de la Universidad Nacional Autónoma de México. Serie Zoología* 1998;69:23-37.
3. Rojas-Molina N, Pedraza-Sánchez S, Torres-Bibiano B, Meza-Martínez H, Escobar-Gutiérrez A. Gnathostomosis, an emerging disease in Acapulco, Mexico. *Emerg Infect Dis* 1999;5:264-6.
4. Vargas OF, Alarcón RE, Alvarado AFJ. Human gnathostomiasis in Mexico. *Int J Dermatol* 1998;37:433-53.
5. Diego P, Lozada L. Estudios florísticos de Guerrero No. 3. Laguna de Tres Palos. Facultad de Ciencias, Universidad Nacional Autónoma de México; México, D.F., 1994. p. 29.
6. Margolis L, Esch GW, Holmes JC, Kuris AM, Schad GA. The use of ecological terms in parasitology (Report of an ad hoc committee of the American Society of Parasitologists). *J Parasitol* 1982;68:131-3.
7. Almeyda AJ. Hallazgo de *Gnathostoma binucleatum* n. sp. (Nematoda: Spirurida) en felinos silvestres y el papel de peces dulceacuícolas y oligohalinos como vectores de la gnathostomiasis humana en la cuenca baja del Río Papaloapan, Oaxaca-Veracruz, México. *Anales del Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México* 1991;18:137-55.
8. Lamothe-Argumedo R, Medina-Vences R, López-Jiménez S, García-Prieto L. Hallazgo de la forma infectiva de *Gnathostoma* sp., en peces de Temascal, Oaxaca, México. *Anales del Instituto de Biología de la Universidad Nacional Autónoma de México. Serie Zoología* 1989;60:311-20.

## First Report of Human Granulocytic Ehrlichiosis from Southern Europe (Spain)

**To the Editor:** Human granulocytic ehrlichiosis (HGE) is a tickborne zoonosis described in the United States several years ago (1) and in Europe recently (2). Several hundred cases

have been reported in the United States (3). In Europe, nine cases have been reported, six in Slovenia (2,4-6), and three in Sweden (I. Eliasson, <http://www.healthnet.org/programs/promed.html>). We report a serologically confirmed case of HGE in La Rioja, a Lyme disease-endemic area in northern Spain (7-9).

On August 7, 1999, a 16-year-old man from La Rioja, who had been bitten by a tick 15 days before, was seen in an emergency room and treated with 100 mg of doxycycline twice a day. On August 9, he was hospitalized with a 3-day history of malaise, myalgias, headache, and fever (39°C). The fever abated in the next 36 hours. The patient had not noticed any signs of inflammation or skin rash, and no signs of neurologic injury were evident. He had abdominal pain when the liver was palpated. Chest radiographs were normal, and abdominal ultrasonography showed no abnormalities. Laboratory studies showed a level leukocyte count (3,001/mm<sup>3</sup> [normal range, 4,500-11,000] with 4.3% band forms, 72.3% neutrophils, 4.7% monocytes, 16.7% lymphocytes, and a platelet count of 114,000/mm<sup>3</sup> [normal, 160,000-410,000]). The hemoglobin level was normal. No inclusions (morulae) suggestive of *Ehrlichia* or *Babesia* spp. were seen on blood smears. The erythrocyte sedimentation rate was normal. The aspartate aminotransferase level was 72 U/L [normal, 5-40]; alanine aminotransferase, 65 U/L [normal, 5-40]; and lactodehydrogenase, 637 U/L [normal, 100-250]. All serologic assays were performed by the same, widely experienced microbiologist, in one laboratory. Serologic test results were negative for *Borrelia burgdorferi* (by enzyme-linked immunosorbent assay [ELISA]); *Rickettsia conorii* (indirect fluorescent-antibody assay [IFA]); *Coxiella burnetii* (IFA); *Ehrlichia chaffeensis* (IFA); the agent of HGE (IFA); and hepatitis A, B, and C viruses (ELISA); and indicated immunity for Epstein-Barr virus. Four weeks later, the aminotransferase levels were normal, and the patient was asymptomatic. A new serum determination showed an HGE antibody titer of 1:64 (HGE IFA IgG MRL Diagnostics, California, USA); the serum tested negative for the other microorganisms tested, including with a new test for *E. chaffeensis*. Another serum sample from the patient taken 8 weeks later showed a titer of 1:256 to the HGE

agent. An EDTA-treated sample of whole blood obtained from the patient on day 4 after start of doxycycline treatment was negative for the *E. phagocytophila* genogroup by polymerase chain reaction (PCR). We used a set of primers based on the published sequence of the 16s rRNA of *E. phagocytophila* (E1: 5'- GGC ATG TAG GCG GTT CGC TAA GTT - 3' and E2: 5'- CCC CAC ATT CAG CAC TCA TCG TTT A -3') (7). Multiple water samples and a positive blood sample from an experimentally infected lamb were used as controls for PCR amplicon contamination. Doxycycline was administered for 14 days, and the patient's clinical and laboratory abnormalities resolved.

Many tickborne diseases are present in La Rioja. The prevalence of *E. phagocytophila* genogroup in the tick *Ixodes ricinus* is high (24.1% of nymphs, determined by PCR) in La Rioja, and evidence of HGE infection in patients at risk has been reported (10,11). This patient's history of previous tick bite, flulike symptoms, seroconversion to HGE agent, aminotransferase elevation, and response to doxycycline suggest the diagnosis of HGE. As in other reported cases in Europe, no morulae suggestive of *Ehrlichia* infection in the acute phase were visible, the clinical manifestations were moderate, and the fever abated quickly with treatment. Also, as in other cases, the negative PCR result can be explained by the prior treatment with doxycycline.

**José A. Oteo,\* José R. Blanco,\*  
Victor Martínez de Artola,\*  
and Valvanera Ibarra\***

\*Hospital de La Rioja, Logroño, Spain

## References

1. Chen SM, Dumler JS, Bakken JS, Walker DH. Identification of a granulocytotropic *Ehrlichia* species as the etiologic agent of human disease. *J Clin Microbiol* 1994;32:589-95.
2. Petrovec M, Furlan SL, Zupanc TA, Strle F, Brouqui P, Roux V, et al. Human disease in Europe caused by a granulocytic *Ehrlichia* species. *J Clin Microbiol* 1997;35:1556-9.
3. McQuiston JH, Paddock CD, Holman RC, Childs JE. The human ehrlichioses in the United States. *Emerg Infect Dis* 1999;5:635-42.
4. Weber R, Pusterla N, Loy M, Lutz H. Fever, leukopenia, and thrombocytopenia in a patient with acute Lyme borreliosis were due to human granulocytic ehrlichiosis. *Clin Infect Dis* 1998;26:253-4.

5. Lotric-Furlan S, Petrovec M, Zupanc TA, Nicholson WL, Summer JW, Childs JE, et al. Human granulocytic ehrlichiosis in Europe: clinical and laboratory findings for four patients from Slovenia. *Clin Infect Dis* 1998;27:424-8.
6. Laferl H, Hogrefe W, Köck T, Pichler H. A further case of acute human granulocytic ehrlichiosis in Slovenia. *Eur J Clin Microbiol Infect Dis* 1999;18:385-6.
7. Oteo JA. Ticks: a century as vector. *Rev Clin Esp* 1995;195:11-2.
8. Oteo JA, Backenson PB, del Mar Vitutia M, García-Moncó JC, Rodríguez I, Escudero R, et al. Use of CRH/he Lyme disease mouse model for the recovery of a Spanish isolate of *Borrelia garinii* from erythema migrans lesions. *Res Microbiol* 1998;149:39-46.
9. Oteo JA, Martínez de Artola V, Eiros JM. Human ehrlichiosis in Spain. Some epidemiological considerations. *Enferm Infecc Microbiol Clin* 1995;13:265.
10. García-Pérez AL, Mandaluniz N, Barral M, Juste RA. Microscopic and PCR findings in sheep after experimental infection with *Ehrlichia phagocytophila*. *Small Ruminant Res* 1999. In press 2000.
11. Oteo JA, Blanco JR. Human granulocytic ehrlichiosis. An emerging zoonosis in our medium? *Enferm Infecc Microbiol Clin* 1999;17:267-8.

## Phylogenetic Analysis of the Chinese *Rickettsia* Isolate BJ-90

**To the Editor:** Five species of tick-associated rickettsiae have been identified in China; of these, three are human pathogens and two are of unknown pathogenicity (1). In 1990, one isolate, BJ-90, was first obtained from a *Dermacentor sinicus* tick, a newly recognized vector collected in a Beijing suburb, an atypical location for *Rickettsia sibirica* (2). Several taxonomic studies of the phenotype, antigenicity, and genotype of BJ-90 have been performed, with inconsistent results (2-6). Recently, phylogenetic analysis based on several gene comparisons has enabled the phylogenetic classification of this rickettsial species (7-11). To confirm the phylogenetic relationships between the BJ-90 strain and other rickettsiae, the 16S rRNA, *gltA*, and *OmpA* encoding genes were amplified and sequenced. Phylogenetic relationships between the BJ-90 strain and other rickettsia in the GenBank database were inferred by the parsimony and neighbor-joining methods (9). Bootstrap analyses were used to assess the reliability of the phylogenetic analysis.

Both methods showed a high degree of similarity between BJ-90, *R. sibirica* and "*R. mongolotimonae*," which were grouped in the same cluster in three inferred dendrograms. The data from the 16S rRNA and *gltA* sequences showed low statistical significance in the cluster (bootstrap values for the nodes 50% and 33%, respectively). However, data from the *ompA* gene sequence showed highly significant similarity in the cluster (bootstrap value 100%), confirming the reliability of the phylogenetic analysis. The results of this phylogenetic analysis are consistent with those of previous phenotypic, genotypic, and phylogenetic analyses (2,3,5-11), as well as taxonomy derived from direct antigenic comparison of the species (4). The sequences of 16S rRNA, *gltA*, and *OmpA* have been assigned the following GenBank accession numbers: AF178036 for 16S rRNA, AF178035 for *gltA*, AF179365 for the 611-bp sequence of *ompA*, and AF179367 for the 3174-bp sequence of *ompA*. According to previous genotypic and antigenic studies and our phylogenetic analysis, in which the BJ-90 strain is closer to *R. sibirica* than *R. mongolotimonae* in the dendrogram inferred from comparison of the *ompA* encoding gene sequences, the BJ-90 strain should be considered a variant of *R. sibirica*.

**Jian Z. Zhang,\*† Min Y. Fan,† Xue J. Yu,‡ and D. Raoult\***

\*Faculté de Médecine, Marseille, France;

†Chinese Academy of Preventive Medicine, Beijing, P.R. China; University of Texas Medical Branch, Galveston, Texas, USA‡

## References

1. Fan MY, Zhang JZ, Chen M, Yu XJ. Spotted fever group rickettsioses in China. In: Raoult, D, Brouqui P, editors. *Rickettsiae and rickettsial diseases at the turn of the third millennium*. Paris: Elsevier; 1999. p. 247-57.
2. Yu XJ, Fan MY, Bi DZ. Primary identification of spotted fever group *Rickettsiae* isolated from *Dermacentor sinicus* in the Beijing area. *Chin J Microbiol Immunol* 1991;11:305.
3. Yu XJ, Jin Y, Fan MY, Xu GM, Liu QH, Raoult D. Genotypic and antigenic identification of two new strains of spotted fever group *Rickettsiae* isolated from China. *J Clin Microbiol* 1993;31:83-9.
4. Xu WB, Raoult D. Taxonomic relationships among spotted fever group *Rickettsiae* as revealed by antigenic analysis with monoclonal antibodies. *J Clin Microbiol* 1997;36:887-96.